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Real-life use of remdesivir-containing regimens in COVID-19: a retrospective case-control study

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SUMMARY

Background: Remdesivir (REM) has shown potent antiviral activity *in vitro* and efficacy in animal models of COVID-19; nevertheless, clinical trials and real-life reports have shown conflicting data on its effectiveness. Aims of the study were to evaluate the impact of remdesivir on I) Intensive Care Unit (ICU) admission, II) need for orotracheal intubation (OTI) and III) in-hospital mortality. Furthermore, we estimated the kinetics of laboratory parameters and assessed the risk factors for in-hospital mortality in the remdesivir population.
Methods: We conducted a retrospective, single-center, case-control (1:1) study including hospitalized patients with confirmed SARS-CoV-2 infection. Cases were patients treated with remdesivir for 5 days, controls were patients not receiving remdesivir.
Results: A total of 192 patients (96 cases and 96 controls) were included in the study. Patients receiving remdesivir had a lower rate of ICU admission and need for OTI than controls, whereas no difference between cases and controls were observed as for mortality rate. However, at multivariable analysis remdesivir was not associated with ICU admission neither with OTI. Instead, presence of haematological malignancies, lower duration of symptoms, higher severity of infection and low lymphocytes count at admission were independently associated with in-hospital mortality. In patients treated with remdesivir a low albumin value and duration of lymphopenia were significantly associated with mortality.
Conclusions: Our real-life study showed that therapy with remdesivir did not have impact on either ICU admission, need for OTI or in-hospital mortality.

Keywords: COVID-19, remdesivir, antiviral therapy, lymphopenia, real-life study.

INTRODUCTION

The novel coronavirus disease 2019 (COVID-19) pandemic, caused by a newly emerging severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is still ongoing and has become a global health crisis [1]. Up to February 8 2022, almost 400 million people have been infected worldwide with more than 5.7 million deaths [2] . At the time of writing, few vaccines have been approved and there is still no effective antiviral treatment for SARS-CoV-2 [3].
In the current health emergency, several therapeutic agents have been evaluated for the prevention and treatment of COVID-19, but the definition of an efficacious drug remains still challenging. Remdesivir (GS-5734) is a nucleotide analogue prodrug that inhibits viral RNA-dependent RNA polymerase which was firstly developed to treat Ebola and further demonstrated an *in-vitro* inhibitory activity against coronaviruses, including also SARS-CoV-2 [4-7]. Furthermore, *in-vivo* study on SARS-CoV-2 infected macaques found that an early remdesivir (REM) treatment reduced clinical disease and damage to the lungs [8].

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Based on these findings, REM has been proposed as a promising option for treating hospitalized patients and, afterwards, it was the first antiviral drug approved from the FDA for the treatment of COVID19 [9]. Nevertheless, clinical trials have shown conflicting data on its effectiveness. The World Health Organization (WHO) SOLIDARITY therapeutics trial, the largest published RCT of REM so far, showed little or no effect on hospitalized patients with COVID-19 compared with standard of care [10]. Instead, the second major RCT of REM ACTT-1, sponsored by the National Institutes of Health (NIH), found a lower time to recovery compared with placebo-controls [11].
Still, in order to predict REM effectiveness and safety in daily clinical practice, it is important to complement the results from RCTs with an evaluation of their transferability to a real-life setting. Also in these settings, the data are conflicting, even if few studies have been published so far [12-16].
Aim of the study was to evaluate the effect of REM-containing regimens on I) Intensive Care Unit (ICU) admission, II) need for orotracheal intubation (OTI) and III) in-hospital mortality in patients infected with SARS-CoV-2. Furthermore, we estimated the kinetics of several laboratory parameters during hospitalization and assessed the risk factors of in-hospital mortality only in patients treated with REM.

PATIENTS AND METHODS

Over an 8-month period (May-December 2020), we conducted a single-center, retrospective, case-control (1:1) study including hospitalized patients with confirmed SARS-CoV-2 infection by polymerase-chain-reaction assay, at Policlinico Umberto I, Sapienza University of Rome. Cases were patients treated with REM-based regimens, controls were patients not receiving REM-based regimens. During the study period, the most common viral variant Italy, was the wild type genome Wuhan-Hu-1 [17].
REM-containing regimens included REM plus at least one of the followings: steroids, enoxaparin, azithromycin, hydroxychloroquine and tocilizumab; whereas controls included regimens containing at least one of the followings: steroids, enoxaparin, azithromycin, hydroxychloroquine and tocilizumab. Cases and controls were matched for age (10-year interval), sex, duration of symptoms before hospitalization (days) and severity of infection at hospital admission (expressed by PaO2/FiO2). Following the national guidelines available at the time, REM was used in patients with radiologic evidence of pneumonia and oxygen support [18]. In detail, REM was used in patients hospitalized from October 2020, when the drug started to be available in our hospital. Controls were patients that met the exclusion criteria for REM or that were hospitalized before October 2020. Exclusion criteria for REM use included alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels greater than 5 times the upper limit of the normal range, estimated creatinine clearance less than 30 ml per minute (by the Cockcroft–Gault formula) or duration of symptoms more than 10 days. According to local protocol, all the case-patients received 200 mg of REM on day 1 as loading dose, followed by 100 mg once daily for the subsequent 4 days as maintenance dose, for a total of 5 days of treatment. For each patient, laboratory, clinical and radiological data at admission and during hospitalization, as well as treatment data and SARS-CoV-2 RNA time of detection, were collected and recorded anonymously in an electronic database. As for treatment data, the use of steroids or tocilizumab was based on clinical judgement and on the national and local guidelines available at that time. Moreover, the use of tocilizumab was also based on drug availability in our hospital at that time. To explore the effect of REM-based regimens on our study population, we collected three major outcomes: ICU admission, the need for OTI and in-hospital mortality.
Furthermore, to investigate the characteristics and outcomes of the whole REM-population during the hospitalization, we systematically collected laboratory analyses, and respiratory features at days 0 (before REM), 6 and 10 after first REM dose, respectively.
The study was approved by the local Ethics Committee (ID Prot. 109/2020).

Statistical analysis

The data were given as medians with interquartile ranges (IQR, 25th-75th percentile) for continuous variables and as simple frequencies, proportions, and percentages for categorical variables. Mann–Whitney test was used for unpaired

samples. Dichotomous variables were compared using Fisher's exact tests or chi-square test statistics, as appropriate. Differences in laboratory parameters over time (d0-d6-d10) were evaluated by Wilcoxon test. For lymphopenia, an absolute count of 725 cells/mmc was used, according to previous reports [19]. Log-rank test and univariate Cox regression were used for categorical or continuous variables, respectively. Multivariable Cox regression model was performed to tease out the independent predictors of in-hospital mortality whereas the need for OTI and ICU admission were evaluated with univariable and multivariable logistic regression. Spearman correlation analyses between duration of corticosteroids (days) and duration of viral shedding (days) as well as duration of viral shedding (days) and duration of symptoms were also performed. P-value analyses were two-sided and a p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed with SATA/IC software (StataCorp) version 15.

RESULTS

Case-control study population

In the study period, we included a total of 109 patients treated with REM and systematically followed up at days 0-6 and 10. Amongst the whole REM population, 13 patients were further excluded due to the absence of matched controls (Figure 1). Therefore, a total of 192 patients (96 cases, 96 controls) were studied, 58 females and 134 males with a median age of 64 years (56-72). Demographic and clinical characteristics of the study population are shown in Table 1. The median PaO_2/FiO_2 at admission was 279 (211-336.5) while the median duration from symptoms onset to hospitalization was 6 days (3-8).

No differences between cases and controls were observed regarding age, sex, severity of infection (expressed by PaO2/FiO2) and duration of symptoms before hospitalization, reflecting the adequacy of the match. Cases had lower levels of D-dimer than controls [579.5 (382.2-1175.5) vs 965 (608-1939) µg/L, *p*: 0.002] and higher platelet count [213 (165-290) vs 197 (162-242) x10^9/L, *p*: 0.04]. Types of symptoms were similar, with the exception of headache (17% vs 1% in cases and controls, *p*: 0.001, respectively).

With regard to therapy, in the REM-containing group 93 patients (97%) received also steroids, 74 (77%) azithromycin, 95 (99%) enoxaparin and only 1 (1%) tocilizumab, while in the control group 31 (32%) patients received steroids, 44 (46%) azithromycin, 74 (77%) hydroxychloroquine, 40 (42%) tocilizumab and 71 (74%) enoxaparin (Table 1).

Patients receiving REM-containing regimens had a lower rate of ICU admission than controls (6.2% vs 20.8%, *p*: 0.003). Univariate analyses showed that male sex, higher severity of infection, low lymphocytes, high C-reactive protein (CRP) and the presence of haematological malignancies were associated with ICU admission whereas receiving REM was protective. Since corticosteroids use showed a trend on lower rate of ICU admis-

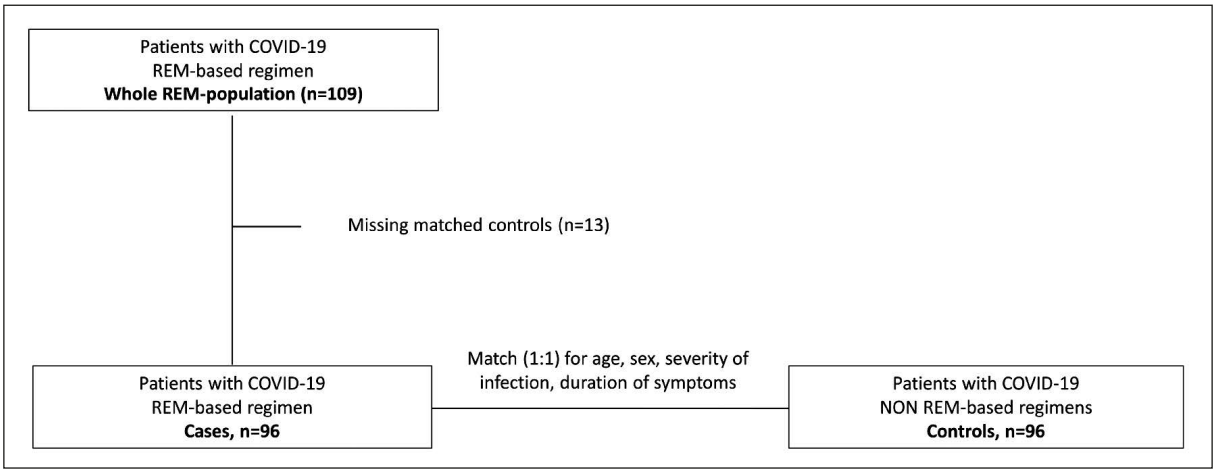

Figure 1 - Flow-chart of the study population.

Table 1 - Demographic and clinical characteristics of the study population.

Characteristics	*Total*	*REM (n=96)*	*NO-REM (n=96)*	*p -value*
Demographics				
Age, years, median (IQR) †	64 (56-72)	64 (56-72)	64 (56-72)	0.797
Gender, F/M †	58/134	29/67	29/67	1.0
Comorbidities, n. (%)				
Hypertension	88 (45.8)	47 (49)	41 (42.7)	0.472
Cardiac arrhythmias	14 (7.3)	8 (8.3)	6 (6.2)	0.565
Diabetes	29 (15)	10 (10.4)	19 (19.8)	0.074
Coronary artery disease	18 (9.4)	7 (7.3)	11 (11.4)	0.321
Heart failure	16 (8.3)	8 (8.3)	8 (8.3)	0.804
Cerebrovascular disease	12 (6.2)	6 (6.2)	6 (6.2)	0.77
Chronic Obstructive Pulmonary Disease	19 (9.9)	6 (6.2)	13 (13.5)	0.086
Asthma	7 (3.6)	3 (3.1)	4 (4.2)	0.711
Obesity (BMI>30)	22 (11.4)	16 (16.6)	6 (6.2)	**0.013**
Dyslipidemia	32 (16.6)	21 (21.9)	11 (11.4)	0.289
Lymphoma/leukaemia	10 (5.2)	5 (5.2)	5 (5.2)	0.9
Cancer, ‡	15 (7.8)	7 (7.3)	8 (8.3)	0.983
Clinical features, n. (%)				
Fever	169 (88)	84 (87.5)	85 (88.5)	0.267
Cough	89 (46.3)	37 (38.5)	52 (54.2)	0.052
Dyspnoea	106 (55.2)	50 (52.1)	56 (58.3)	0.724
Fatigue	37 (19.3)	14 (14.6)	23 (24)	0.547
Dysgeusia/anosmia	26 (13.5)	17 (17.7)	9 (9.4)	0.183
Diarrhoea	26 (13.5)	15 (15.6)	11 (11.4)	0.382
Headache	18 (9.4)	17 (17.7)	1 (1)	**0.001**
Days from symptoms onset to hospitalization, median (IQR) †	6 (3-8)	6 (4-7.5)	6,9 (2.8-9.6)	0.399
Laboratory findings at the admission, median (IQR)				
WBC, x 10^6/L	6765 (4832.5-9052.5)	7070 (5250-9015)	6055 (4757.5-9070)	0.377
PMN, x 10^6/L	4945 (3333-7672,5)	5450 (3655-7610)	4620 (3250-7750)	0.606
LYM, x 10^6/L	870 (650-1185)	885 (705-1205)	820 (565-1180)	0.862
PMN/LYM	5 (3.1-9.4)	4 (4-8)	5.2 (2.9-9.8)	0.225
PLTs, x 10^9/L	208 (162-257)	213 (165-290)	197 (162-242)	*0.04*
Albumin g/L	3.7 (3.3-4)	3.6 (3.3-3.9)	3.7 (3.2-4.1)	0.649
D-dimer, µg/L	787 (461.5-1696)	579.5 (382.2-1175.5)	965 (608-1939)	**0.002**
CRP, mg/dL	5.2 (2.7-9.8)	4.9 (2.7-9.0)	5.6 (2.5-11.5)	0.381
Respiratory features				
PaO2/FiO2 ratio at the admission, median (IQR) †	279 (211- 336.5)	269 (204.5-330.5)	286 (217.2-350)	0.121
Non-invasive ventilation with HFNC/CPAP, n. (%)	109 (56.8)	57 (58.4)	52 (54.2)	0.519

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Characteristics	*Total*	*REM (n=96)*	*NO-REM (n=96)*	*p -value*
Treatment				
Corticosteroids, n. (%)	121 (63)	93 (96.9)	28 (29.2)	**<0.0001**
Days of CCS-therapy, median (IQR)	14 (10-20)	15 (11-21)	11 (10-18.5)	**0.002**
Remdesivir, n. (%)	96 (50)	96 (100)	0 (0)	NA
REM therapy start < 5 days of symptoms onset, median (%)	23 (23.9)	23 (23.9)	0 (0)	NA
Outcomes				
ICU admission, n. (%)	26 (13.2)	6 (6.2)	20 (20.8)	**0.003**
Orotracheal intubation, n. (%)	16 (8.3)	4 (4.2)	12 (12.5)	**0.028**
Mortality, n. (%)	27 (14.1)	10 (10.4)	17 (17.7)	0.146
Days of hospitalization, median (IQR)	19 (16-26.2)	20 (14-25)	16 (12-27)	0.964
Days of viral shedding, median (IQR)	18 (12-25)	21 (16.7-28.5)	14.5 (10-21.5)	**<0.0001**

† characteristics used in the match; ‡ cancer in the last 5 years.
Abbreviations: IQR: interquartile range I-III; *BMI:* Body Mass index; *WBC:* white blood cells; *PMN:* polymorphonuclear leukocytes; *LYM:* lymphocytes; *PMN/LYM:* polymorphonuclear leukocytes and lymphocytes ratio; *PLTs:* platelets; *CRP:* C-reactive protein; *HFNC:* High Flow Nasal Cannula; *CPAP:* Continuous Positive Airway Pressure; *CCS:* corticosteroids; *REM:* remdesivir; *ICU:* Intensive Care Unit; *NA:* Not applicable.

Table 2 - Multivariable analyses. Factors associated with ICU Admission (panel A), need for orotracheal intubation (panel B) in- hospital mortality (panel C), in case-control population and mortality in the whole REM-population (panel D).

A.		
	OR (95% CI)	*p value*
Gender (female)	0.44 (0.11-1.72)	0.239
PaO2/FiO2	0.99 (0.987-0.998)	**0.015**
REM	0.37 (0.09-1.48)	0.159
LYM	0.999 (0.998-1.00)	0.218
CRP	1.00 (1.00-1.00)	**0.006**
Leukaemia/Lymphoma	7.75 (1.59-37.82)	**0.011**
Steroids	0.48 (0.13-1.79)	0.272
B.		
	OR (95% CI)	*p value*
PaO2/FiO2	0.99 (0.98-1.00)	**0.038**
REM	0.78 (0.13-4.76)	0.789
Steroids	0.19 (0.03-1.06)	0.058
LYM	6.29 (1.29-30.77)	**0.023**
Low Albumin	1.53 (0.41-5.65)	0.523
Leukaemia/Lymphoma	6.52 (1.35-32.54)	**0.022**

C.		
	OR (95% CI)	*p value*
Age	1.03 (0.98-1.09)	0.265
Days of symptoms	0.82 (0.70-0.96)	**0.012**
PaO2/Fio2	0.99 (0.98-0.99)	**0.001**
LYM	0.99 (0.996-0.999)	**0.036**
CRP	1.00 (0.999-1.000)	0.449
Leukaemia/Lymphoma	28.87 (4.33-192.27)	**0.001**
REM	0.65 (0.15-2.81)	0.564
Steroids	0.40 (0.08-1.87)	0.243
Enoxaparin	1.43 (0.17-11.86)	0.743
D.		
	OR (95% CI)	*p value*
Age	1.03 (0.11-1.72)	0.597
Gender	0.19 (0.987-0.998)	0.193
REM start <5d	7.78 (0.09-0.1.48)	0.082
Low Albumin	0.62 (0.998-1.0)	**0.016**
D-dimer	1.00 (1.0-1.0)	0.455
Lymphopenia > 6d	56.48 (1.59-37.82)	**0.002**

Abbreviations: REM: remdesivir; *LYM*: low lymphocytes count (<725 cells/mmc); *CRP*: C-reactive protein; *REM start <5d*: Remdesivir started within five days of the onset of symptoms; *Lymphopenia >6d*: lymphocytes count lower than 725 cells/mmc for more than 6 days).

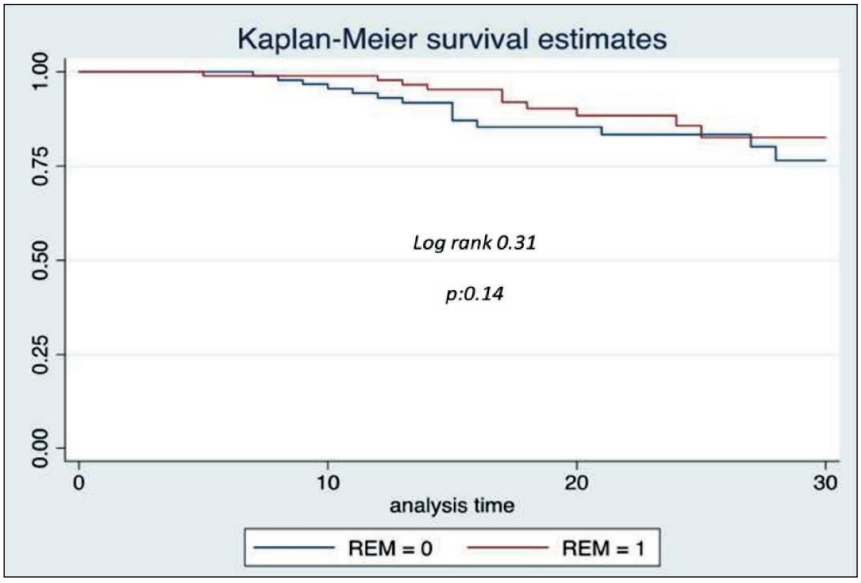

Figure 2 - Cumulative proportion of mortality rate between patients who received REM (red line) and patients who did not received REM (blue line).

sion (*p*: 0.06), in the final multivariable model we decided to insert also this variable. However, at multivariable analysis only high CRP (*p*: 0.006), severity of infection (*p*: 0.015) and haematological malignancies (*p*: 0.011) were independently associated with ICU admission (area under the curve, AUROC, of the model 0.84) (Table 2.A).

Likewise, patients receiving REM had a lower rate of need for OTI (4.2% vs 12.5%, *p*: 0.028) but in the multivariable analysis no significant association was observed between cases and controls on the need for OTI (Table 2.B), whereas the use of steroids showed a trend towards protection from OTI (*p*:0.058).

As for in-hospital mortality, no significant differences between cases and controls were observed (10.4% vs 17.7%, *p*: 0.14, respectively) (Figure 2). Univariate analyses showed that older age, lower duration of symptoms, higher severity of infection, low lymphocytes, high CRP and the presence of haematological malignancies were associated with mortality. No association with mortality was found for the use of corticosteroids (*p*: 0.35) or REM (*p*: 0.15). At multivariable analysis, lower duration of symptoms at the hospital presentation (*p*: 0.012), higher severity of infection (*p*: 0.001), low lymphocytes (*p*: 0.036) and the presence of haematological malignancies (*p*: 0.001) were independently associated with mortality (AUROC 0.91) (Table 2.C).

Viral shedding

Overall, median duration of viral shedding was 18 days (IQR 12-25), 21 days (IQR 16.25-28.75) in REM group versus 14.5 days (IQR 10-21.75) in subjects not receiving REM ($p<0.0001$). Of note, a statistically significant positive correlation between duration of viral shedding (days) and duration of corticosteroids treatment was found (r 0.6220, $p<0.0001$) (Figure 3). On the other hand, duration of viral shedding and duration of symp-

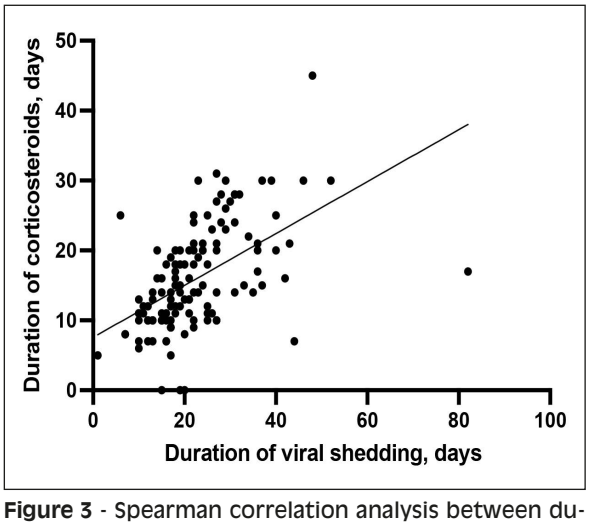

Figure 3 - Spearman correlation analysis between duration of viral shedding (days) and duration of corticosteroids treatment (days) (r 0.6220, p<0.0001).

toms before hospitalization did not show correlation (r:-0.03, *p*: 0.64).

Characteristics of REM-containing population

As for the whole REM population, we recollected data from all the 109 hospitalized adult patients (age >18 years) diagnosed with SARS-CoV-2 pneumonia that received REM-based regimen for 5 days (Figure 1) and that were followed up with laboratory and respiratory parameters at days 0-6-10. In Table 3 the general characteristics of the population and the variation from d0 to d10 of different laboratory parameters are depicted.

Patients treated with REM showed a significant increase of lymphocytes B, $CD4^+$ and $CD8^+$ T-cells count from day 0 to day 10 of therapy (Figure 4A-B-C-D), as well as D-dimer values, while CRP and IL-6 constantly decreased during treatment

Table 3 - Remdesivir population. Clinical and laboratory characteristics of the whole population treated with remdesivir-containing regimens.

General characteristics	*N=109*		
Age, years, median (IQR)	62 (55-71)		
Gender, females/males	29/67		
Days of symptoms onset to hospitalization, median (IQR)	6 (4- 8)		
Days of hospitalization, median (IQR)	20 (14-26)		
Days of viral shedding, median (IQR)	22 (17-29)		
pO2 at the admission, median (IQR)	89 (73.6-113)		
PaO2/FiO2 at the admission, median (IQR)	269 (204.5-330.5)		
Outcomes			
At least one episode of bradycardia, n. (%)	34 (31.2)		
ICU admission, n. (%)	6 (6.2)		
Orotracheal intubation, n. (%)	5 (5.2)		
In-hospital mortality, n. (%)	10 (10.4)		
Laboratory analysis, median (IQR)	*d0 †*	*d6*	*d10*
WBC, x 10^6/L	7260 (5290-9060)*	8350 (6585-10050)^	8760 (7700-10360)**
PMN, x 10^6/L	5840 (3690-7670)*	5885 (4450-7835)	6080 (5070-7950)**
LYM, x 10^6/L	880 (710-1180)*	1310 (865-1910)^	1580 (1120-2340)**
Mono, x 10^6/L	340 (250-450)*	480 (375-640)	520 (410-660)**
PMN/LYM	4.25 (4-8.5)	4 (2-7)^	4.06 (2.46-6.27)**
PLTs, x10^9/L	211 (166-268)*	308 (229-402)	282 (224-387)**
CRP, mg/dL	5.21 (2.8-9.42)*	1.54 (0.72-4.22)^	0.42 (0.2-1.18)**
IL-6, pg/mL	31.1 (15.24-65)*	12.02 (6.10-23.59)^	6.98 (3.31-21.1)**
Serum procalcitonin, ng/ml	0.09 (0.04-0.17)*	0.06 (0.04-0.115)	0.05 (0.03-0.117)**
Albumin, g/L	3.6 (3.3-3.9)*	3.4 (3.1-3.7)	3.5 (3.2-3.8)**
Ferritin, µg/L	718 (336-1209)	726 (425-1300)^	586 (346 -899)**
Lactate dehydrogenase, U/L	267 (224-338)	232 (205-284)	214 (177-244)
Creatinine, mg/dL	0.86 (0.72-1.01)*	0.84 (0.7-0.96)^	0.84 (0.74-0.99)
AST, U/L	26 (20-35)	28 (19-37)^	20 (15-34)**
ALT, U/L	24.5 (16-38.5)*	28 (19-37)	20 (15-34)**
Fibrinogen, mg/dL	544 (443-599)*	437.5 (366.5-510.5)^	397 (322-487)**
D-dimer, µg/L	583 (388-1017)*	680 (419-1229)^	556 (318-1354)

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Lymphocyte counts, median (IQR)			
Lymphocyte T CD4, #	436.5 (382-609)*	731(483-1113)^	988 (712-1281)**
Lymphocyte T CD4, %	42.2 (35.4-54)*	48.6 (42.9-56.4)^	49.7 (42.1-57.9)**
Lymphocyte T CD8, #	182 (118-279)*	278 (161-406)^	324 (204-460)**
Lymphocyte T CD8, %	19 (13.7-26.9)*	17. 4 (13.4-27.1)^	16.6 (13.2-23)**
Lymphocyte B cell, #	139.5 (89-200)*	279 (148-422)^	310 (185-503)**
Lymphocyte B cell, %	14.5 (11.2-21.5)*	19.1 (12.1-24.5)^	17.6 (12-22.2)**
Respiratory parameters, median (IQR)			
pO2	87 (73-113)*	109 (85-137)	110 (85-128)**
pCO2	34 (32-37.2)	36 (33-40)	37 (34-40)
PaO2/FiO2	256 (196-320)	243 (176-326)^	259 (180.5-351)
sO2	98.8 (97-99.4)	99.5 (98.6-100)	99.6 (98.4-99.9)
Non-invasive ventilation with HFNC/CPAP, n. (%)	19 (17.4)*	41 (37.6)^	20 (18.3)**
Orotracheal intubation, n. (%)	0	0	1 (0.9)

†d: days from first REM dose.
Abbreviations: IQR: interquartile range I-III; *ICU*: Intensive Care Unit; *WBC*: white blood cells; *PMN*: polymorphonuclear leukocytes; *LYM*: lymphocytes; *Mono*: monocytes; *PMN/LYM*: polymorphonuclear leukocytes and lymphocytes ratio; *PLTs*: platelets; *CRP*: C-reactive protein; *IL-6*: interleukin-6; *AST*: aspartate aminotransferase; *ALT*: alanine aminotransferase; *HFNC*: High Flow Nasal Cannula; *CPAP*: Continuous Positive Airway Pressure. *: statistically significant (p<0.05) between d0-d6; ^statistically significant (p<0.05) between d6-d10; **: statistically significant (p<0.05) between d0-d10.

(Figure 4). Of note, these parameters did change over time in survived patients whereas no difference was found in deceased patients (Figure 4). Regarding the early use of REM, 23 (23.9%) patients started therapy within 5 days of symptoms onset. Looking at the risk factors for mortality in the REM-population, a low albumin value at admission (*p*: 0.016) and a low lymphocytes count (<725 cells/mmc) for more than 6 days (*p*: 0.002) were significantly associated with a worse outcome (Table 2.D) while no effect was observed for the early REM therapy (*p*: 0.082).

DISCUSSION

In the present study, we showed that REM-containing regimens did not have effect on ICU admission, need for OTI and mortality, although in the univariate analyses REM represented a protective factor for OTI and ICU admission.
Currently, an optimal implementation of therapeutic regimens aimed to decrease COVID-19 morbidity and mortality is a global dare. REM effectiveness is still debated and this drug has been a subject of controversy since RCTs ACTT-1 and SOLIDARITY have been published. Indeed, WHO international COVID-19 guidelines recommend against its use, while NIH guidelines recommend REM with a moderate rating, especially in patients that need oxygen support [20, 21]. Even though few RCT results showed a shorter time to clinical improvement, REM seems to not have any effect on mortality rate and few or no impact on clinical benefit [10, 11, 22-24]. Similarly, in our retrospective case-control study, the REM-based regimens were not associated with improvement in the mortality rate. These data are in agreement with the results published by Wang et al and those observed in the SOLIDARITY study, that is, still, the largest, clearest and simplest RCT available so far [24, 25].
Conflicting data were observed also in the real-life experiences on REM published at the time of writing. In fact, our findings are in agreement with *Garibaldi* et al and *Ohl* et al (2021), that showed absence of impact on mortality in their case-control cohort, while *Lee* and colleagues (2020) reported a higher mortality rate in patients receiving REM compared with previous RCTs [12, 13, 15]. In contrast, a Spanish descriptive study in a cohort of 242 patients admitted for COVID-19 in the 2020, among which 123 were treated with REM, observed an overall low mortality rate [16]. Likewise, *Diaz* and colleagues (2021) confirmed

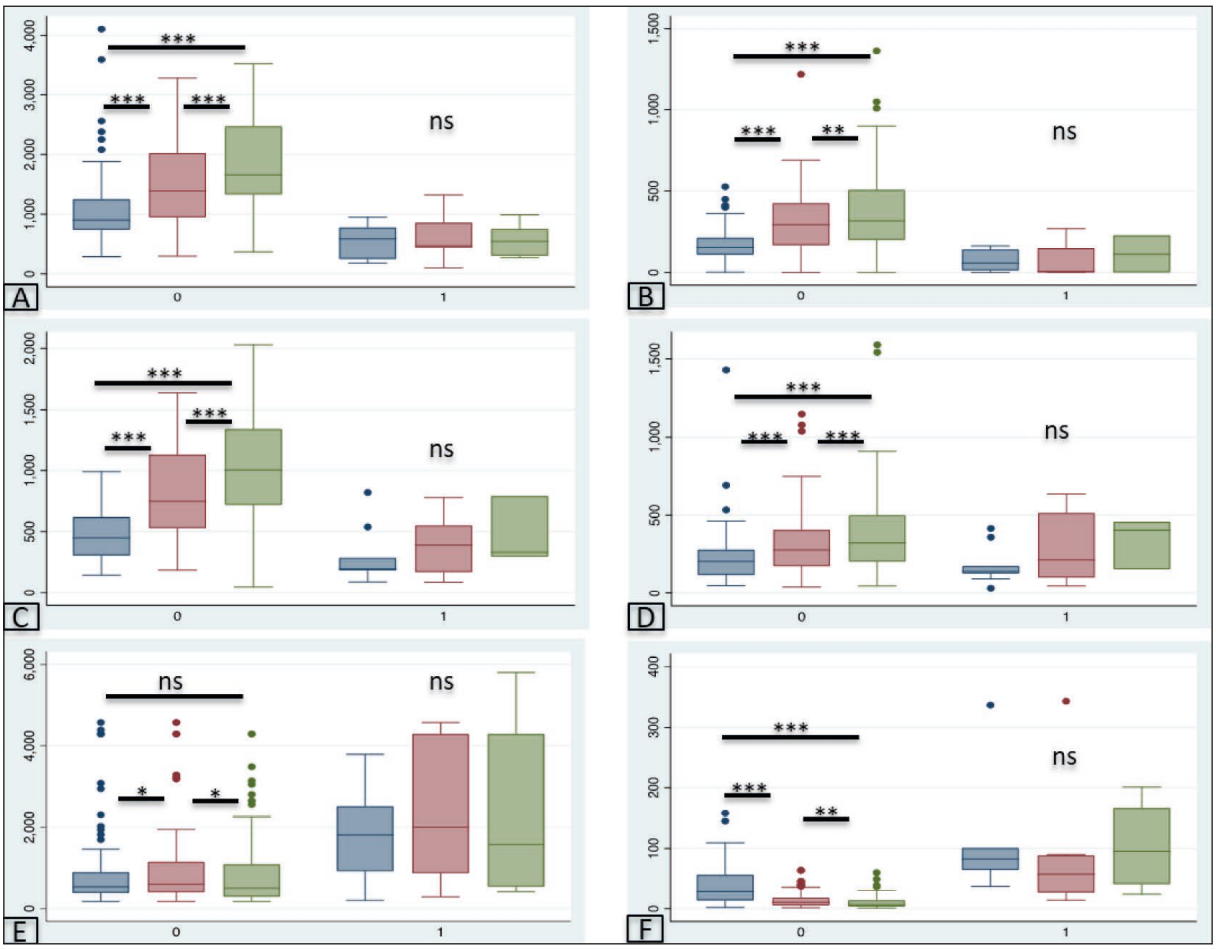

Figure 4 - Time variation of different laboratory analyses from day 0 to day 10 in the whole REM population.
Panel A: total lymphocytes count (cells/mmc); Panel B: lymphocytes B (cells/mmc), Panel C: $CD4^+$ T-cells (cells/mmc), Panel D: $CD8^+$ T-cells (cells/mmc), Panel E: D-dimer value (μg/L), Panel F: IL-6 value (pg/ml). "0" refers to survided patients; "1" refers to died subjects. Blue column: d0; red column: d6; green column d10. *: p: 0.05-0.01, **: p<0.01, ***: p<0.001; ns: not significant.

a lower mortality rate in patients receiving REM compared to those receiving the best supportive care [26]. Moreover, a recent real-life experience including 285 patients treated with REM matched with as many not receiving it, showed a significant decrease in the time to clinical recovery among patients admitted to the hospital for COVID-19 and treated with REM, although the mortality rate was not affected by receiving the drug [12].

The observed divergence amongst published studies could be explained by their retrospective nature and the heterogeneous methods employed to recollect and report data. In view of all these discordant results from RCTs and real-life reports, the decision to use or not REM as a part of treatment should take into account the therapy cost-effectiveness that may exceed the benchmark, as it has been previously reported [27]. Anyway, we strongly believe that additional studies with higher number of patients are warranted.

Exploring the risk factors related with mortality in our case-control population, we found that lower duration of symptoms at the hospital admission and the presence of haematological malignancies were independently associated with mortality. While the first observation may be related to a more severe presentation of COVID-19 pneumoniae at hospital admission, especially during

the first pandemic wave, the latter confirms that patients with haematological malignancies represent a vulnerable population to worse outcome during COVID-19 due to a prolonged and severe immunosuppression [28, 29].

In the present study, viral shedding was significantly longer in cases than in controls. Few data have been published on the effects of REM on viral load and viral shedding, with no data available from RTCs and only a real-life evaluation observing no nasopharyngeal viral load changes [30]. Recently, a faster viral clearance in patients treated with REM plus steroid compared to patients only treated with steroids was observed, thus assuming a direct REM role on viral shedding [31]. Our different findings may be explained by the significant difference in steroids use between cases and controls. As a matter of fact, receiving steroids represents a determinant factor of prolonged viral shedding, not only during COVID-19 but also during other viral infections including seasonal influenza, SARS and MERS [32]. Furthermore, we found a statistically significant positive correlation between duration of viral shedding and duration of corticosteroids treatment confirming the central role of steroid therapy on the viral shedding, as it has been already observed [32]. On the other hand, the duration of viral shedding did not seem correlated to other factors as symptoms duration before hospitalization.

Regarding the timing of REM use, we did not observe any impact on mortality starting therapy within 5 days of symptoms onset. This finding is apparently in contrast with the last published RCT PINETREE, which showed that a short (3-day) and early REM regimen prevents progression to severe COVID-19 by means of hospitalization and mortality reduction [33]. Nevertheless, these results could not be matched for the different nature of the studies design and the more severe COVID-19 presentation of our study population. We strongly consider that studies reporting the real-life use of early REM in outpatients and inpatients are needed.

An interesting finding of the present report is the duration of lymphopenia as an independent biomarker of mortality in patients treated with REM. In fact, lymphopenia has been already associated with mortality in patients affected by community-acquired pneumonia caused by viral and bacterial aetiologies [34]. As a matter of fact, in influenza virus infection lymphopenia was associated with a more severe course of disease and, similarly, several studies have suggested how lymphopenia could be a predictor of poor prognosis even in COVID-19 patients [35-37]. However, an association between duration of lymphopenia in COVID-19 and mortality in literature is still lacking and, according to the result of our report, having a low lymphocyte count (*i.e.* <725 cells/mmc) for more than 6 days represents an independent risk factor for an adverse outcome. Additional studies on the prognostic role of the duration of low lymphocyte count should be therefore encouraged.

Furthermore, low albumin value was an independent risk factor for mortality in patients treated with REM-based regimens. This result is in line with several observations showing that hypoalbuminemia was associated with worse outcome and coagulopathy in COVID-19 patients [38, 39].

Last, but not least, in our study population REM therapy was well tolerated, since no severe adverse events have been reported. Nevertheless, we observed that a not-negligible rate of patients experienced at least one episode of bradycardia during or after REM-therapy, in the absence of other factors potentially influencing the heart rate (*i.e.* beta-blockers). Although COVID-19 may be *per se* associated with bradycardia, probably due to a direct viral effect, also REM use has been commonly associated with an increased risk of transient bradycardia [40-42]. How REM could be responsible for bradycardia onset is still unknown; however, in line with its pharmacodynamics properties, an effect on sinoatrial node function might be suggested [42].

This study has several limitations. In fact, firstly a generalization about the results cannot be done due to the small sample of patients and the monocentric and retrospective nature of the study. Secondly, data regarding time to recovery were not collected; nevertheless, considering the retrospective nature of the study, gathering this information could have led to significant bias when collecting data. Thirdly, no typing was performed on the SARS-CoV-2 genome detected in COVID-19 patients hospitalized. Finally, although regimens between cases and controls were sometimes different and the cases were more likely to be treated with steroids, the extensive match as well as the inclusion of corticosteroids in the final multivariable model contributed to the reduction of potential bias.

In conclusion, our real-life study showed that therapy with REM did not have effect on in-hospital mortality but suggested that REM may have a potential role on OTI and ICU admission, although these findings were not confirmed at multivariate analysis. Length of steroid therapy was significantly correlated with the duration of viral shedding in the whole population and the duration of lymphopenia was independently associated with mortality in patients treated with REM.

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Conflict of interest
The authors declare that there are no conflicts of interest.

Authors' contributions
Study design: Cogliati Dezza Francesco, Oliva Alessandra, Claudio Maria Mastroianni; data collection: Mauro Vera, Romani Francesco Eugenio, Aronica Raissa, Savelloni Giulia, Casali Elena, Valeri Serena, Cancelli Francesca; data analysis: Cogliati Dezza Francesco, Oliva Alessandra; writing-original draft preparation: Cogliati Dezza Francesco; writing-review and editing: Oliva Alessandra, Claudio Maria Mastroianni; supervision: Oliva Alessandra, Claudio Maria Mastroianni. All authors have read and agreed to the published version of the manuscript.
Cogliati Dezza Francesco and Oliva Alessandra equally contributed to the manuscript.

REFERENCES

[1] World Health Organization, 'A year without precedent: WHO's COVID-19 response', Dec. 23, 2020. Available at: https://www.who.int/news-room/spotlight/a-year-without-precedent-who-s-covid-19-response [accessed 08 Feb. 2022].
[2] Johns Hopkins University, 'COVID-19 Map', *Johns Hopkins Coronavirus Resource Center*, 2021. Available at: https://coronavirus.jhu.edu/map.html [accessed 08 Feb. 2022].
[3] European Medicines Agency, 'COVID-19 vaccines', *European Medicines Agency*, Mar. 19, 2021. Available at: https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/coronavirus-disease-covid-19/treatments-vaccines/covid-19-vaccines [accessed 08 Feb. 2022].
[4] Malin JJ, Suárez I, Priesner V, et al. Remdesivir against COVID-19 and other viral diseases. *Clin. Microbiol. Rev.* 2020; 34 (1). doi: 10.1128/CMR.00162-20.
[5] Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. *Sci. Transl. Med.* 2017; 9 (396). doi: 10.1126/scitranslmed.aal3653.
[6] Yin W, Mao C, Luan X et al. Structural basis for inhibition of the RNA-dependent RNA polymerase from SARS-CoV-2 by remdesivir. *Science*. 2020; 368 (6498), 1499-504. doi: 10.1126/science.abc1560.
[7] Wang M, Cao R, Zhang L, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res.* 2020; 30 (3), 269-71. doi: 10.1038/s41422-020-0282-0.
[8] Williamson BN, Feldmann F, Schwarz B, et al. Clinical benefit of remdesivir in rhesus macaques infected with SARS-CoV-2. *Nature*. 2020; 585 (7824), 273-76. doi: 10.1038/s41586-020-2423-5.
[9] The U.S. Food and Drug Administration, 'FDA Approves First Treatment for COVID-19', *FDA*, Oct. 22, 2020. Available at: https://www.fda.gov/news-events/press-announcements/fda-approves-first-treatment-covid-19 [accessed 08 Feb. 2022].
[10] WHO Solidarity Trial Consortium et al. Repurposed antiviral drugs for Covid-19 - Interim WHO Solidarity Trial Results. *N. Engl. J. Med.* 2021; 384 (6), 497-511. doi: 10.1056/NEJMoa2023184.
[11] Beigel JH, Tomashek KM, Dodd LE, et al. Remdesivir for the treatment of Covid-19 - final report. *N. Engl. J. Med.* 2020; 83 (19),1813-26. doi: 10.1056/NEJMoa2007764.
[12] Garibaldi BT, Wang K, Robinson ML, et al. Comparison of time to clinical improvement with vs without remdesivir treatment in hospitalized patients with COVID-19. *JAMA Netw. Open*. 2021; 4 (3), e213071. doi: 10.1001/jamanetworkopen.2021.3071.
[13] Ohl ME, Miller DR, Lund BC, et al. Association of Remdesivir treatment with survival and length of hospital stay among US veterans hospitalized with COVID-19. *JAMA Netw. Open*. 2021; 4 (7), e2114741. doi: 10.1001/jamanetworkopen.2021.14741.
[14] Frost MT, Jimenez-Solem E, M. Ankarfeldt MZ, et al. The Adaptive COVID-19 Treatment Trial-1 (ACTT-1) in a real-world population: a comparative observational study. *Crit. Care*. 2020; 24, 677. doi: 10.1186/s13054-020-03406-3.
[15] Lee S, Santarelli A, Caine K, et al. Remdesivir for the treatment of severe COVID-19: a community hospital's experience. *J. Osteopath. Med.* 2020; 120 (12), 926-33. doi: 10.7556/jaoa.2020.156.
[16] Garcia-Vidal C, Meira F, Cózar-Llistó A, et al. Real-life use of remdesivir in hospitalized patients with COVID-19. *Rev. Espanola Quimioter.* 2021. 34 (2), 136-40. doi: 10.37201/req/018.2021.
[17] Lai A, Bergna A, Menzo S, et al. Circulating SARS-CoV-2 variants in Italy, October 2020–March 2021. *Virol. J.* 2021; 18, 168. doi: 10.1186/s12985-021-01638-5.

[18] Agenzia Italiana del Farmaco, 'Farmaci utilizzabili per il trattamento della malattia COVID19', Nov. 24, 2020. Available at: https://aifa.gov.it/aggiornamento-sui-farmaci-utilizzabili-per-il-trattamento-della-malattia-covid19 [accessed 08 Feb. 2022].
[19] Oliva A, Ceccarelli G, Borrazzo C, et al. Comparison of clinical features and outcomes in COVID-19 and influenza pneumonia patients requiring intensive care unit admission. *Infection*. 2021; 49 (5), 965-75. doi: 10.1007/s15010-021-01624-7.
[20] Agarwal A, Rochwerg B, Lamontagne F, et al. A living WHO guideline on drugs for covid-19. *BMJ*. 2020; 370, m3379. doi: 10.1136/bmj.m3379.
[21] National Institute of Health, 'COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines', *COVID-19 Treatment Guidelines*, Jul. 08, 2021. Available at: https://www.covid19treatmentguidelines.nih.gov/management/clinical-management/hospitalized-adults--therapeutic-management/ [accessed 208 Feb. 2022].
[22] Spinner CD, Gottlieb RL, Criner GJ, et al. Effect of remdesivir vs standard care on clinical status at 11 days in patients with moderate COVID-19: a randomized clinical trial. *JAMA* 2020; 324 (11), 1048-57. doi: 10.1001/jama.2020.16349.
[23] Olender SA, Perez KK, Go AS, et al. Remdesivir for Severe COVID-19 versus a cohort receiving standard of care. *Clin Infect Dis.* 2021; 73 (11), e4166-e74. doi: 10.1093/cid/ciaa1041.
[24] Wang Y, Zhang D, Du G, et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *The Lancet*. 2020; 395 (10236), 1569-78. doi: 10.1016/S0140-6736(20)31022-9.
[25] Dyer O. Covid-19: Remdesivir has little or no impact on survival, WHO trial shows. *BMJ*. 2020; 371, m4057. doi: 10.1136/bmj.m4057.
[26] Diaz GA, Christensen AB, Pusch T, et al. Remdesivir and mortality in patients with COVID-19 [published online ahead of print, 2021 Aug 19]. *Clin Infect Dis*. 2021; ciab698. doi: 10.1093/cid/ciab698
[27] Institute for Clinical and Economic Review, 'ICER Provides Second Update to Pricing Models for Remdesivir as a Treatment for COVID-19', *ICER*, Nov. 10, 2020. Available at: https://icer.org/news-insights/press-releases/icer-provides-second-update-to-pricing-models-for-remdesivir-as-a-treatment-for-covid-19/ [accessed 22 Jul. 2021].
[28] García-Suárez J, de la Cruz J, Cedillo Á, et al. Impact of hematologic malignancy and type of cancer therapy on COVID-19 severity and mortality: lessons from a large population-based registry study. *J Hematol Oncol.* 2020; 13 (1), 133. doi: 10.1186/s13045-020-00970-7.
[29] El-Sharkawi D, Iyengar S. Haematological cancers and the risk of severe COVID-19: Exploration and critical evaluation of the evidence to date. *Br J Haematol.* 2020; 190 (3), 336-45. doi: 10.1111/bjh.16956.
[30] Goldberg E, Ben Zvi H, Sheena L, et al. A real-life setting evaluation of the effect of remdesivir on viral load in COVID-19 patients admitted to a large tertiary centre in Israel. *Clin. Microbiol. Infect.* 2021; 27 (6), 917. doi: 10.1016/j.cmi.2021.02.029.
[31] Marrone A, Nevola R, Sellitto A, et al. Remdesivir plus dexamethasone versus dexamethasone alone for the treatment of COVID-19 patients requiring supplemental O2 therapy: a prospective controlled non-randomized study [published online ahead of print, 2022 Jan 27]. *Clin Infect Dis.* 2022; ciac014. doi:10.1093/cid/ciac014
[32] Cogliati Dezza F, Oliva A, Cancelli F, et al. Determinants of prolonged viral RNA shedding in hospitalized patients with SARS-CoV-2 infection. *Diagn. Microbiol. Infect. Dis.* 2021; 100 (2), 115347. doi: 10.1016/j.diagmicrobio.2021.115347.
[33] Gottlieb RL, Vaca CE, Paredes R et al. Early Remdesivir to prevent progression to severe Covid-19 in outpatients. *N Engl J Med*. 2022; 386 (4), 305-15. doi: 10.1056/NEJMoa2116846.
[34] Méndez R, Menéndez R, Amara-Elori I, et al. Lymphopenic community-acquired pneumonia is associated with a dysregulated immune response and increased severity and mortality. *J. Infect.* 2019. 78 (6), 423-31. doi: 10.1016/j.jinf.2019.04.006.
[35] Bellelli V, d'Ettorre G, Celani L et al. Clinical significance of lymphocytopenia in patients hospitalized with pneumonia caused by influenza virus. *Crit. Care*. 2019; 23, 330. doi: 10.1186/s13054-019-2608-1.
[36] Tan L, Wang Q, Zhang D et al. Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study. *Signal Transduct. Target. Ther.* 2020; 5 (1), 1-3. doi: 10.1038/s41392-020-0148-4.
[37] Ziadi A, Hachimi A, Admou B et al. Lymphopenia in critically ill COVID-19 patients: A predictor factor of severity and mortality. *Int. J. Lab. Hematol.* 2021; 43 (1), e38-40. doi: 10.1111/ijlh.13351.
[38] Violi F, Ceccarelli G, Cangemi R et al. Hypoalbuminemia, Coagulopathy, and Vascular Disease in COVID-19. *Circ. Res.* 2020; 127 (3), 400-01. doi: 10.1161/CIRCRESAHA.120.317173.
[39] Violi F, Cangemi R, Romiti GF et al. Is albumin predictor of mortality in COVID-19?. *Antioxid. Redox Signal.* 2020; 35 (2), 139-42. doi: 10.1089/ars.2020.8142.
[40] Oliva A, Franchi C, Gatto MC et al. Prevalence and clinical significance of relative bradycardia at hospital admission in patients with coronavirus disease 2019 (COVID-19). *Clin. Microbiol. Infect.* 2021; 27 (8), 1185-87. doi: 10.1016/j.cmi.2021.04.013.
[41] Pallotto C, Blanc P, Esperti S et al. Remdesivir treatment and transient bradycardia in patients with coronavirus diseases 2019 (COVID-19). *J. Infect.* 2021; 83 (2), 237-79. doi: 10.1016/j.jinf.2021.05.025.
[42] Touafchia A, Bagheri H, Carrié D et al. Serious bradycardia and remdesivir for coronavirus 2019 (COVID-19): a new safety concern. *Clin. Microbiol. Infect.* 2021; 27 (5), 791. doi: 10.1016/j.cmi.2021.02.013.